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Insecticide resistance study in *Plutella xylostella* L.

I. Developing a sampling method for surveying[1]

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Abstract : A sampling method for surveying the insecticide-resistance in diamondback moth, *Plutella xylostella* L. has been developed in a large vegetable growing area in Taiwan. We identified a diamondback moth population which was homogeneous in the permethrin resistance and the coverage of this population was about five thousand hectares. The result indicated that the diamondback moth has the ability in mixing their different genetic strains in a quite large area. The fact made us believe that the distribution pattern survey of insecticide-resistance in diamondback moth served a real significance in the future pest management study of this pest. Based on the analysis of the data obtained, we further considered that sampling diamondback moth in five separated cruciferus fields in an area of five thousand hectares was both economical and accurate in measuring the insecticide resistance.

Alarmed by rapid increase in registered insecticides and the development in insecticide-resistance in diamondback moth, *Plutella xylostella* L. [6,8,9], Taiwan Agricultural Research Institute has initiated a resistance survey program ; its purpose being to evaluate the present status of insecticidal control of the cruciferus pests. A detailed map of resistance distribution pattern is most desirable for this purpose but it can not be prepared without designing a suitable sampling method for it. In this study, we have tried to develop an economical and accurate sampling method to fulfill our need. Recently, Liu et al [7] reported that diamondback moth had developed resistance to synthetic pyrethroids in most of vegetable growing area. We intended to select an important vegetable producing area to be our model for studying and developing the sampling method. Permethrin, a synthetic pyrethroid, is our model insecticide for resistance test. Besides, we have selected cartap, an insecticide with different mode of action from permethrin, as a comparing compound.

Materials and Methods

Site selection : Hsihu-Yungchien was chosen as the location for our model in studying

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insecticide resistance survey. Because it is the largest vegetable producing district in Taiwan ; vegetables are grown year-round and insecticides are constantly and intensively used. We divided the whole area into four regions from northwest to southeast and the distance from region to region is about four to five kilometers. At the center of each region, we sampled four spots and each sampling spot is an independent cruciferus field, the distance between two sampling spots is one hundred to two hundred meters. A detailed map of regions and sampling spots is shown in Fig. 1 ; the letter (A to D) indicates

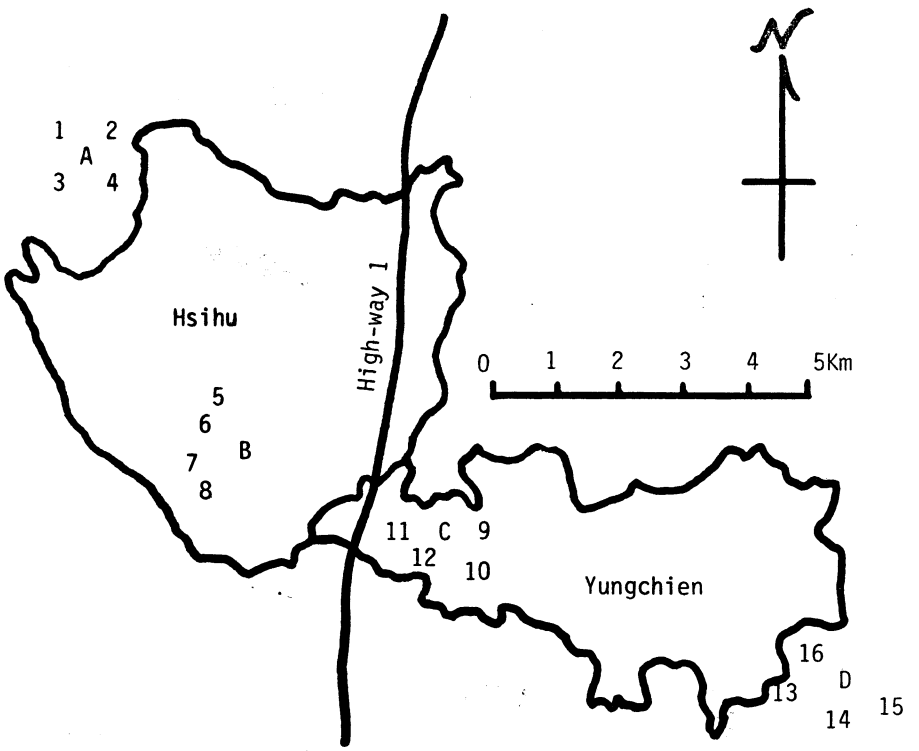

Fig. 1. The detailed map of sampling location and regions in Hsihu-Yungchien area.

the region and the number (1 to 16) represents the sampling spot.

Insects and insecticides : In each sampling spot, we collected two hundred to three hundred diamondback moth larvae or pupae. Insects were brought back to the laboratory and were reared on insecticide-free cabbage to the adult stage ; the adults were fed with honey-water mixture daily. After mating, females oviposited their eggs on a potted cabbage in the screen cage. Only the third instar larvae of F_1 generation of collected specimen were used in the insecticide LC_{50} test, and the insecticide treatment was conducted in a Potter Spray Tower. Cartap and permethrin were chosen to test all sixteen samples and both chemicals were obtained in 95% technical grade from industrial suppliers.

Cartap was dissolved in a mixture of acetone and water (1 : 1, v/v) and permethrin was dissolved in acetone only. In spraying operation, one milliliter of insecticide solution was first pipetted into the holding tube of Tower, then we switch on the spray control,

the solution was sucked into a stainless steel U-tubing and sprayed out from the nozzle into the tower. After the spray, we waited for ten seconds before lowering the stage to take out the objects. The holding tube was rinsed throughly two to three times with a proper solvent whenever we changed the insecticide or the concentration. In the permethrin test, insects were put on a sheet of paper and sprayed with insecticide directly, then they were released on a piece of fresh cabbage leaf in a petri dish. A total of eleven different concentrations of permethrin ranged from 1,200ppm to 11,700ppm were used in the test. In the cartap test, insects were not sprayed with insecticide directly; instead they were fed with a piece of throughly sprayed cabbage leaf in a petri dish. Likewise, eleven different concentrations of cartap ranged from 150ppm to 6,000ppm were used. Ten to fifteen third-instar diamondback moth larvae were treated in one replica and results of three replicas were sumed together for probit analysis. The insect mortality of each treatment was counted twenty-four hours after the spray; the post-treatment holding temperature and relative humidity were controlled at 28±1.5°C and 80±5% respectively.

Analysis : The toxicity data in LC_{50} of all samples were subjected to the F-test for examining the significant difference among regions. Since the arrangement of regions were relative in a line which extended from A to D, the analysis also followed that order. If the difference among region A to D was significant, further tests were carried out on region A to C and on region B to D separately, and this procedure was continued until the coverage of a homogeneous population was identified. Since the chemical control intensity of sampling spot may influence the population density of natural enemies, the rate of parasitism was examined and tabulated according the sample location. The parasitism data were subjected to the same analysis procedure as the toxicity data.

Results

The insecticide sensitivity of diamondback moth, *Plutella xylostella* L., from Hsihu-Yungchien to permethrin and cartap are assembled in Table 1 and 2 respectively. Field

Table 1. The permethrin susceptibility of *Plutella xylostella* sampled from four regions in Hsihu-Yungchien area.

Field	LC_{50} of sampled *P. xylostella* (ppm)			
	A	B	C	D
1	3,820	—[a]	5,830	2,415
2	2,653	7,662	4,560	4,854
3	6,207	5,223	4,837	3,590
4	6,533	7,646	4,836	3,143
Average[b]	4,803	6,844	5,016	3,501

a. not enough insects for the LC_{50} test.

b. the laboratory strain has a LC_{50} value at 93 ppm.

Table 2. The cartap susceptibility of *Plutella xylostella* sampled from four regions in Hsihu-Yungchien area.

Field	LC_{50} of sampled *P. xylostella* (ppm)			
	A	B	C	D
1	520	649	796	623
2	560	506	547	687
3	336	299	503	320
4	503	421	703	704
Average[a]	480	469	637	583

a. the laboratory strain has a LC_{50} value at 345 ppm.

strains of diamondback moth have significantly higher LC_{50} value than the laboratory strain in the permethrin test and the range varied from 26 to 82 times. These results confirmed that the diamondback moth population in Hsihu-Yungchien had developed resistance to permethrin which is a rather universal phenomenon in the whole area, in spite of the fact that the level of resistance might be different from region to region as our analysis later indicated.

In the cartap test, the difference of insecticide susceptibility between the field strains and the laboratory strain was not comparable to the permethrin test. It was either that cartap resistance had not yet developed in diamondback moth or there was no real susceptible strain at all to compare with. The rate of parasitism of *Apanteles plutellae* in the diamondback moth is presented in Table 3 and those data had through the arcsin transformation prior to the analysis.

Table 3. Per cent of parasitism of *Plutella xylostella* sampled from four regions in Hsihu-Yungchien area.

Field	Per cent of parasitism of *P. xylostella*			
	A	B	C	D
1	19.2	1.8	6.7	31.0
2	10.0	5.8	1.6	16.5
3	19.2	5.6	4.0	39.1
4	6.1	4.0	13.8	19.1
Average[a]	13.6	4.3	6.5	26.4

a. parasitized by *Apanteles plutellae*, the major larval parasite of *P. xylostella* in Taiwan.

The F-test results of the permethrin-susceptibility, the cartap-susceptibility and the rate of parasitism of diamondback moth in regard to region A to D are presented in Table 4; the significant difference level is set at 5%. These results showed that the field

Table 4. The F-test results of the permethrin susceptibility, the cartap susceptibility and the percentage of parasitism of *Plutella xylostella* sampled from four regions in Hsihu-Yungchien area.

Regions	P>F value		
	Permethrin sus.	Cartap sus.	Percent of parasitism[a]
A-B-C-D	0.0429*	0.3191	0.0021*
A-B-C	0.1794	—[b]	0.0655
B-C-D	0.0078*	—[b]	0.0020*

* significant at 5% level.

a. percent of parasitism was tested through the arcsin transformation.

b. further test was not necessary.

strains of diamondback moth from region A to C were homogeneous in both of their permethrin-susceptibility and the rate of parasitism, and all four region's diamondback moth was homogeneous in their captap susceptibility.

In an attempt to examine the relationship between the chemical control intensity and the density of natural enemies in general, we paired the data of Table 1 and 3 for a correlation test. We established a negative correlation between the permethrin-resistance and the rate of parasitism from all samples ; the correlation is significant with the correlation coefficient at -0.57134 and the $P> |R|$ value at 0.025. In order to minimize the sample size to save both time and labor but without critically reducing the information accuracy, we grouped the permethrin-susceptibility data in region A to C and the cartap susceptibility data in the whole area separately as two information pools. Then the

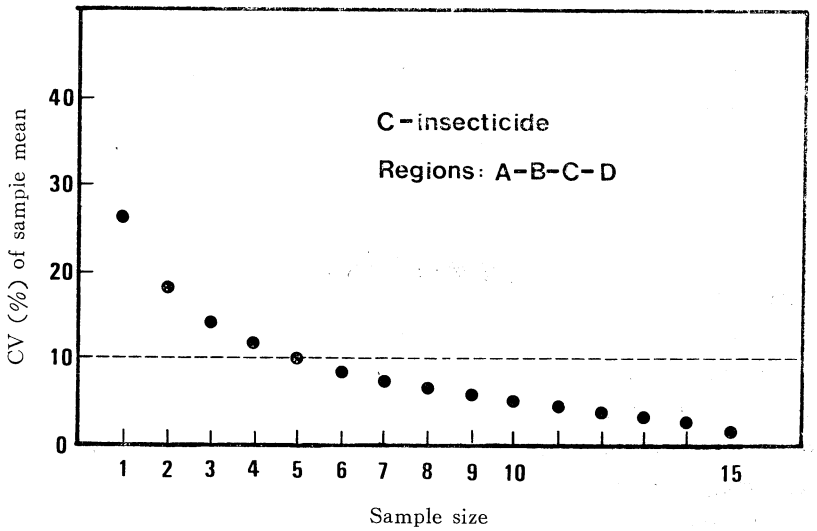

Fig. 2. The relationship of the coefficient of variation (CV) and the sample size from pooled data of cartap susceptibility of *Plutella xylostella* in A-B-C-D region of Hsihu-Yungchien area.

coefficient of variation was tested against the sample size by sampling the information pool and the permethrin data pool containing eleven data and the cartap data pool containing sixteen data. The results were presented in Fig. 2 and 3 ; both tests showed that the coefficient of variation of the sample mean was close or lower than 10% if the sample size increases to five. In other words, by tolerating a coefficient of variation of

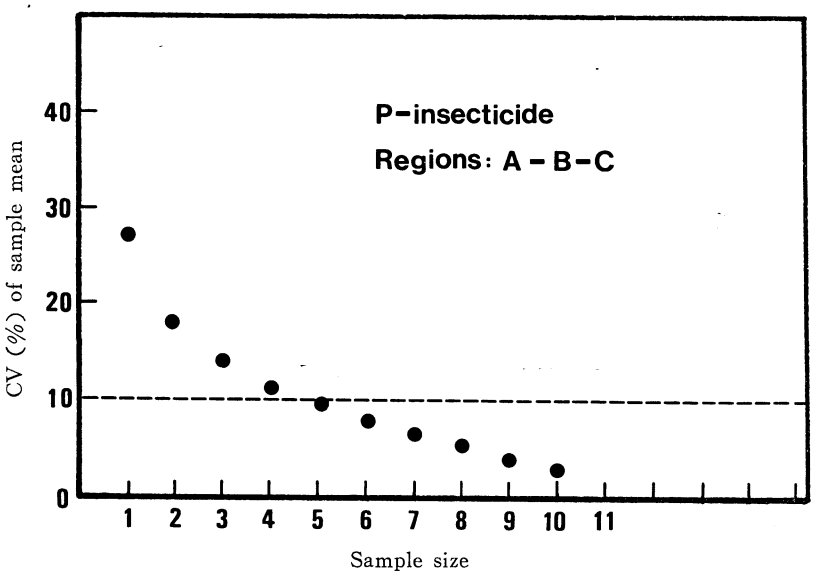

Fig. 3. The relationship of the coefficient of variation (CV) and the sample size from pooled data of permethrin susceptibility of *Plutella xylostella* in A-B-C region of Hsihu-Yungchien area.

sample mean at 10% in both data pools then we can save more than 50 to 68% of time and labor in sampling. Region A to C covers about five thousand hectares and sampling five independent cruciferus fields was adequate in measuring the insecticide-susceptibility of a supposed homogeneous population of the diamondback moth.

The region D was actually surrounded by rice paddy and was not similar to three other regions in the sense of cropping system. The test result confirmed that it's diamondback moth population was less resistance to permethrin than those from other regions.

Discussion

Not only the reports [5,9] but also the field observation in several important vegetable growing areas have convinced us that diamondback moth is the most destructive insect pest on the cruciferus in Taiwan. The constant occurrence of diamondback moth is also a main reason which drives the farmers to adapt an intensive chemical spraying schedule. The early success of chemical control, as it was introduced, has created itself an irreplaceable image for farmers that it is the sole effective measure for the pest control. Even when one insecticide gradually lost its original efficacy, the farmers learned quickly to shift to a new insecticide and not even bother to ask the reason. After twenty years or more in depending only on the chemical control, the diamondback moth

has developed resistance to many insecticides as other investigators reported [1,2,3,4,6,7]. A periodic survey of insecticide resistance in the diamondback moth is urgently needed to identify the troubled regions and their specific problems. Although Chi and Sun [3], Feng and Sun [4], Lee and Lee [6] made field investigations in diamondback moth resistance but none of them had ever designed a sampling method for surveying the distribution pattern of resistance. The study is intended to fulfill the need of an economical and suitable sampling method in surveying a large area. From the results we obtained and the report of Liu et al [7], diamondback moth has clearly developed resistance to permethrin and our data indicated that there are two diamondback moth populations in Hsihu-Yungchien with different permethrin-resistance levels. The first population which covered region A to C has higher resistance than the second population in region D.

Because the coverage of the first population is larger than most of other vegetable concentrated growing areas on the island, developing a proper sampling method in this area will serve well for most of other needed areas as well.

The coverage of first population also indicated that the diamondback moth has the ability in mixing their genetic strains in a quite large area, so surveying the distribution pattern of insecticide resistance in diamonback moth has a real significance in the pest management study.

By examining the overall results, we maintain a general view of our model area i. e., the region A to C is a perennial vegetable cultivating area with high intensity insecticide usage and low natural enemy density. The region D is mostly a rice producing region and the vegetable growing is sporadical, the insecticide usage is less intensive and the natural enemy density is higher than region A to C. The diamondback moth in the whole area has not developed serious resistance to cartap yet, and the homogeneity in their cartap-susceptibility does not necessarily mean that they are homogeneous in other aspects as well. From our analysis, we believe that by sampling diamondback moth in five separate cruciferus fields in an area of five thousand hectares are both economical and accurate in measuring the insecticide resistance.

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小菜蛾抗藥性之研究

I. 調查方法之探討[1]

鄭 允[2]

中文摘要

爲建立本省各蔬菜產地小菜蛾（*Plutella xylostella* L.）抗藥程度測定體系，必須了解小菜蛾抗藥品系空間分布與距離之關係，從而決定正確的取樣距離與頻度。此研究係在溪湖、永靖地區約140平方公里範圍之蔬菜栽培區，每隔四至五公里，設置樣本區一處，全區共設立四個樣本區，每一樣本區取樣四個採集點，各點相距約一至二百公尺，均爲獨立之十字花科菜田，共計十六處採集點，將所採得小菜蛾幼蟲及蛹携返室內，用不噴殺蟲劑之甘藍菜飼養，取其第一代三齡幼蟲，測定其對培丹及百滅靈的半量致死濃度，再將所得資料按區排列，以變方分析不同區間樣本之差異。尋得具共同抗藥程度之族羣後，再比較小菜蛾樣本數與平均值之機差關係，由此一關係訂定最經濟的取樣數，但以不嚴重影響統計值的正確性爲原則，在此，吾人訂定之容許機差爲 10 %，取樣量爲五個樣本點。因爲研究結果顯示抗藥族羣具相同抗藥力者可涵蓋五千公頃之廣，亦小菜蛾有相當的能力不斷地混合田間的生理小種，樣本點相距數百公尺之差異不大，而在無障礙物時此一現象尤其一致，因此在五千公頃範圍之蔬菜專業區內，只需獨立取樣五點，即可測出有代表性的抗藥程度，供預測之用。

1. 臺灣省農業試驗所 研究報告 第996號。本研究爲中央加强農建補助計劃〔70農建-5.1-產-47 (02)〕，承農業發展委員會及省農林廳補助，賴宗賢及周桃美協助研究工作，謹此致謝。
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